JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA), Vol. 5, No. 1, 2020
Chemistry Education Study Program, Universitas Sebelas Maret
https://jurnal.uns.ac.id/jkpk
pp. 131-140
ISSN 2503-4146
ISSN 2503-4154 (online)

THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ENDOPHYTIC FUNGI EXTRACT ASSOCIATED WITH *CHLORANTUS OFFICINALIS* BLUME AND *STAUROGYNE ELONGATA* KUNTZE

Listiana Oktavia[1]*, Evana[1], Muhammad Ilyas[2], Andria Agusta[1]

[1]Research Center for Chemistry, Indonesian Institute of Sciences (LIPI)
Jl. Puspitek serpong Gate, Muncul, South Tangerang City 15314, Banten, Indonesia
[2] Research Center for Biology, Indonesian Institute of Sciences (LIPI)
Jl. Raya Jakarta Bogor Km 46, Muncul, Cibinong, Bogor,16911, West Java, Indonesia

*Correspondance: oktavialisti@gmail.com

Received: May 13, 2020 Accepted: July 12, 2020 Online Published: August 26, 2020
DOI : 10.20961/jkpk.v5i2.40617

ABSTRACT

An endophytic fungi has been widely known for a source of bioactive compounds attributed as antimicrobial and antioxidant. In this report, we investigated the antimicrobial and antioxidant activity of extract of endophytic fungi associated with two indigenous Indonesian medicinal plants, i.e., *Chloranthus officinalis (*CO) and *Staurogyne elongata* (SE). Ten endophytic fungi isolates were collected from barks, roots, and leaves of CO and SE host plants and were cultured into potato dextrose broth (PDB) media. After 14 days of incubation, the whole culture was extracted by ethyl acetate. The qualitative antimicrobial analysis that was conducted by dot blot and TLC-bioautography showed all of the extracts show antimicrobial activity against *Staphylococcus aureus. At the same time,* they were observed less active against *Escherichia coli*. The minimum inhibitory concentration (MIC) analysis showed that endophytic fungi extract of CO_2 and SE3 are classified as strong antibacterial activity against *S. aureus* with MIC value <8 µg/ml. The TLC bioautography of antioxidant displayed the appearance of radical inhibition area from SE4 and SE5 extract. Quantitative antioxidant activity, which was conducted by the radical DPPH scavenging, showed that SE5 has the lowest IC_{50} value, i.e., 56.3679 µg/ml (AAI value 0.5455) and was classified as moderate antioxidant activity. Meanwhile, the remaining extracts are classified as weak antioxidant activity.

Keywords: *Antibacterial, antioxidant, Chloranthus officinalis, endophytic fungi, Staurogyne elongata.*

INTRODUCTION

The fast development of drug resistance microbes and degenerative disease have urged for the discovery of novel and effective therapeutic agents [1, 2]. There are three ways to find the pharmacologically active substances: rational drug design [3], combinatorial chemistry, and natural product discovery [1]. Natural products have been widely known to produce a large variety of biologically active compounds. They represent a vast diversity of fascinating molecule configuration [4]. The discovery of new drug by isolation of bioactive compound from natural resources still get many attentions as approximately 64.9% of the new drugs introduced for nearly four decades (1981-2019) came from natural-product derivatives or modified synthetic inspired by occurring natural products [5].

In decades, plants scientists have started to realize that plants may serve as a source of several endophytes microorganisms [6]. These microbes can live in an intercellular space of plant tissue. Some of these endophytes can be able to produce bioactive substances that have the function of contributing to the host-endophytic relationship [2]. Endophytes form a symbiotic relationship with its plant host. In many cases, the microbes have released secondary metabolites functioned as a biological defense of host plants against phytopathogen [1]. Endophytes have previously reported being able to produce significant therapeutic active compounds such as antimicrobial agents and antioxidants. Other research related to this also reports that about 265 compounds isolated from endophytic fungi that have antimicrobial activity [7]. Endophytic fungi *A. ilanense* associated with Cinnamomum species was reported to produce a group of flavonoids with strong antioxidants activity, including kaempferol, quercetin, genkwanin, and (+)-catechin [8]. The discovery of bioactive compounds from endophytes microbes also can be environmentally friendly as it does not requires a lot of plant sources to get enough amount of extract for investigation. The endophytes microbes can be isolated from the small part of plants and then be in vitro cultured using artificial growth media.

Endophytes are known to be able to produce secondary metabolites that have similar or equal hosts. Bioactive compounds produced or produced together with plants and their endophytes [1]. So thus, in this study, we investigated the antimicrobial and antioxidant activity of the endophytic fungi extract associated with Indonesian traditional medicinal plants *C. officinalis* (Keras tulang) and *S. elongate* (rendeu). *C. officinalis* is traditionally used for tonic and for healing the rheumatic disease. Meanwhile, *S. elongate* is used for the treatment of kidney stone disease. The previous report showed that *S. elongate* has antimicrobial activity against *E. coli* and *S. aureus* [9].

METHODS

1. Materials

a. Plant Source

C. officinalis and *S. elongate* were collected randomly purposive in the Cikaniki Research Station area (6°44' S 106°32' E, 1066 malt). It is surrounding in the area of Mount Halimun-Salak National Park, West Java, and lowland forest area of Bodogol Nature Reserve (6°46' S 106°51' E, 530 malt).

b. Chemicals

Ethanol (70%), NaOCl, glycerol, lactophenol blue, ethyl acetate (Merck), TLC plates (silica gel GF254, Merck), CH_2Cl_2 (Merck), MeOH (Merck) $Ce(SO_4)_2$ (Merck), Iodonitrotetrazolium H_2SO_4 (Merck), chloramphenicol (Merck), Mueller-Hinton broth (MHB, Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma), p-violet (INT, Sigma), and (+)-catechin (Sigma).

2. Research Methods

a. Sample Preparation

Plant samples used using fresh ingredients, and healthy living tissue consists of bark, leaves, and roots. Give a mark to all

fresh and packaged samples and do the packaging correctly and carefully and then taken to the Chemical Bioprospection Research Group, Chemical Research Center-LIPI, in less than 72 hours.

b. Fungal Isolation

The endophytic fungi were isolated based on the surface sterilization method with slight modification [10]. Collected plants were sterilized by sequentially by Ethanol (70%), NaOCl, and distilled water. Small fragments of sterilized plants were then cultured for two weeks at 27 ºC. The growing endophytic fungi were isolated solely by transferring them into potato dextrose agar (PDA) to obtain the pure fungal strain. Purified fungal strains were used for antimicrobial and antioxidant screening and partially restored under inactive metabolism condition with the freezing method at T -80 ºC by used 10% (v/v) glycerol and 5% (g/v) trehalose as a cryoprotectant for the collection purpose [11].

c. Fungal Identification

Mushroom identification is carried out based on a morphological approach. This approach is carried out by observing both microscopic and macroscopic phenotypic characters. Macroscopic characterization is done by following the shape of the colony, color, texture, drop of exudate, surface, and invert the color of fungal culture. Microscopic observation, fungal mycelia by dripping one drop of 1% blue lactophenol solution. Microscopic characterization was carried out with a light microscope to observe hyphae, hyphae pigmentation, clamp connections, spores, septates, and other reproductive structures.

d. Endophytic Fungi Culture Extraction

The identified pure fungal then were cultured in the potato dextrose broth (PDB) and incubated at temperature 27 ^{o}C. After 14 days of incubation, fungal mycelia was taken out from the medium and then smashed mechanically. The slurry fungal mycelia were then extracted with ethyl acetate three times. The solvent was removed from the mixture under reduced pressure. The extracted metabolite was stored in -20 ^{o}C for further investigation.

e. Chemical Compounds Analysis by Thin Layer Chromatography (TLC)

The dried extract was prepared at the concentration of 10 mg/ml in ethyl acetate. The endophytic extract solutions at prepared concentrations were deposited into TLC plates and then were eluted with CH_2Cl_2: MeOH 10:1 (v/v). Eluted chemical compounds were visualized under 254 nm and 366 nm ultraviolet (UV) light and followed by spraying with coloring reagent 1% $Ce(SO_4)_2/H_2SO_4$ and 1% vanillin-sulphuric acid at the elevated temperature [12].

f. The Antibacterial Investigation by TLC-Bioautography

TLC-bioautography guided screening was conducted to observe the antibacterial activity of endophytic fungi extract qualitatively. The extract was transferred into the TLC plate and immersed into bacterial suspensions (*S. aureus* and *E. coli*), which were counted approximately $1x10^8$ CFU. The

treated plates were incubated for 18 hrs at 37°C. INT solution was sprayed to the incubated plates to observe the growth of bacteria. Chloramphenicol was applied as a positive control. Further investigation was applied to the active extracts by eluting the extract with CH_2Cl_2: MeOH (10:1 v/v), and the rest of the procedure is similar to the was written in this section for the bioautography process [13].

g. Minimum Inhibitory Concentration (MIC).

The MIC of all extracts and positive control, chloramphenicol, were determined by the microdilution technique in MHB, for tested bacteria (*S. aureus* and *E. coli*) [14]. The extracts and chloramphenicol were added to MHB plated in the 96-well plate. Serial dilutions were performed to get the final concentrations in order 256, 128, 64, and 32 µg/ml. The tested bacteria (100 µl) was added to each well and were incubated by 18 hrs at 37°C. The INT was added to each well after incubation. The MIC value was determined by the lowest concentration showing the colorless wells.

h. Antioxidant Investigation

A qualitative antioxidant investigation was performed by dot blot technique by transferring extracts and positive control (+)-catechin solution to the TLC plates followed by exposing the plates with DPPH solution in methanol (0.2%). The antioxidant potency was observed 30 minutes after spraying. The yellow-white area observed at the particular extract indicated the antioxidant [13].

The sample concentration inhibits radical activity by 50% (IC50), which is determined by the serial microdilution present in the 96-well microplate. After going through the dilution process, each well is added 100 µl DPPH (61.50 µg / ml). Negative controls are used as negative controls, while (+) - catechins are used as positive controls. The microplates were incubated at room temperature and in the dark for 90 minutes. The absorbance of the sample was carried out at 517 nm. The existing linear regression equation can be used to determine. The antioxidant activity index calculated further classification for antioxidant activity (AAI) formulated as below [13, 15].

$$\mathbf{AAI} = \frac{\mathbf{The\ final\ Cont\ of\ DPPH\ in\ the\ reaction}\ (\hat{A}\mu\mathbf{g/ml})}{\mathbf{IC_{50}}\ (\hat{A}\mu g/ml)}$$

RESULTS AND DISCUSSION

Isolation and Identification of Endophytic Fungi

Table 1. Endophytic fungi from *C. officinalis*

Isolates	Fungal taxa	Host plant	Plant part
CO1	Coelomycetes	*C. officinalis*	Leaf
CO2	*Fusarium* sp.	*C. officinalis*	Bark
CO3	*Xylaria* sp.	*C. officinalis*	Leaf
CO4	*Colletotrichum* sp.	*C. officinalis*	Bark
CO5	*Schizophyllum* sp.	*C. officinalis*	Root
SE1	*Colletotrichum* sp.	*S. elongata*	Bark
SE2	*Colletotrichum* sp.	*S. elongata*	Bark
SE3	Hyphomycetes	*S. elongata*	Bark
SE4	*Colletotrichum* sp.	*S. elongata*	Leaf
SE5	*Colletotrichum* sp.	*S. elongata*	Leaf

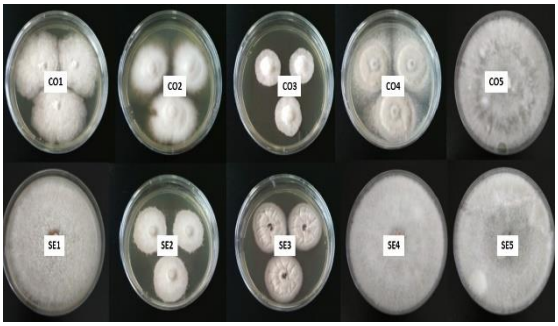
and *S. elongata* plants collected from Cikaniki Research Station, West Java.

A total of ten endophytic fungi were isolated from various parts of *C. officinalis* and *S. elongata*. Based on morphological

characteristics, selected endophytic fungi from *C. officinalis* and *S. elongata* plants were identified as *Fusarium* sp. (1 isolate), *Xylaria* sp. (1 isolate), *Colletotrichum* sp. (5 isolates) and *Schizophyllum* sp. (1 isolate) (Table 1). The other two isolates could only be identified into the class level, i.e., Coelomycetes and Hyphomycetes.

Fungal taxa Coelomycetes and Hyphomycetes are known to have strong associations with higher vascular plants [16]. Many endophytic fungi Hyphomycetes and Coelomycetes are capable of sporulating in their natural habitat or host plant but turn to be sterile or fail to sporulating during in vitro subculturing onto artificial media [17]. The macroscopic view of endophytic fungi cultures is depicted in Figure 1.

Figure 1. Macroscopic picture of fungal isolates.



TLC Analysis of Extracted Isolate

The TLC chromatogram profile of endophytic fungi extracts associated with *C. officinalis* and *S. elongate* plants are given a different chromatogram pattern, as seen in Figure 2. After the TLC plate was sprayed extracts associated with *C. officinalis* and *S. elongata* plants against *S. aureus* and *E. coli* with coloring reagent 1% $Ce(SO_4)_2/H_2SO_4$ and 1% vanillin-sulphuric acid, the formation of different retention factors on multiple stain spots suggest the presence of various chemical compounds in each endophytic fungi extract [18]. The less polar compounds eluted first, while the more polar compounds interacted stronger with the silica, due to the high polarity of the silica compounds as a consequence, the more polar compounds will be eluted later.

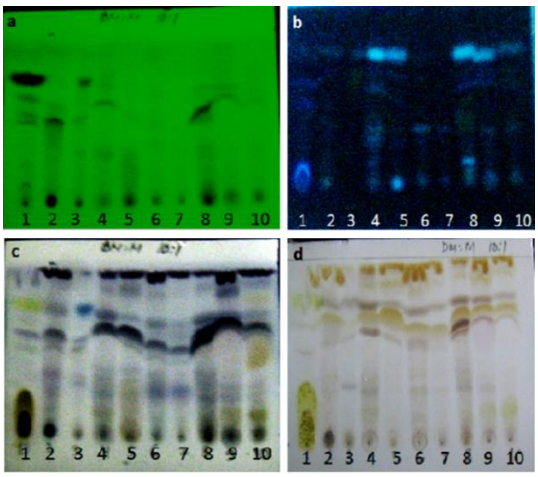


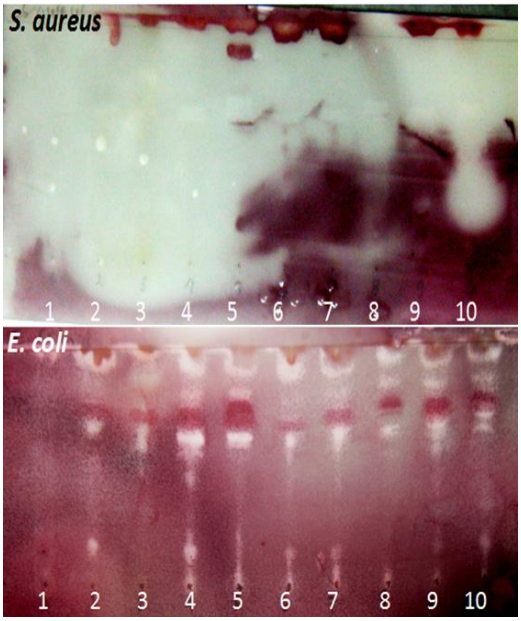
Figure 2. Chromatograms of endophytic fungi extracts developed with an eluent system of CH_2Cl_2:MeOH (10:1), (a) viewed under 254 nm, (b) viewed under 366 nm, (c) sprayed with 1% vanillin-sulphuric acid, and (d) sprayed with 1% cerium (IV) sulfate). Picture information refers to Table 2.

Antibacterial activity

The TLC-bioautography method was carried out for the analysis of the antibacterial activity of endophytic fungi

qualitatively. Figure 3 displays the bioautogram of the antibacterial activity of endophytic fungi extracts.

The results showed that eight endophytic fungi extracts were able to inhibit the growth of *S. aureus* with different inhibitory zone diameters, as shown by the formation of a clear white area around the extracts. In contrast, no extract showed significant activity for *E. coli,* as indicated by the pink area only around the extract. After spraying with the INT solution, the purple color on the TLC plate was caused by the dehydrogenase enzyme reaction in living microorganisms, which converts INT to purple formazan [19].



The positive control used in the antibacterial activity assay was chloramphenicol because of its wide-spectrum activity with MIC range of 1-90 µg/ml [20].

Figure 3. Bioautogram of antibacterial activity of endophytic fungi extracts against *S.aureus* and *E.coli*. Picture information refers to the Table 2.

The extracts were then developed with an eluent system of dichloromethane: methanol (10:1 v/v) to know the chemical substances that are responsible for antibacterial activity in the active extract. The bioautogram of the active antibacterial compounds of the endophytic fungi extracts against *S. aureus* and *E. coli* are depicted in Figure 4.

According to this result, the appearance of white bands in the eluted plates has suggested the presence of more than one active antimicrobial agent. The clear white areas of *S. aureus* were wider than *E. coli*, and it indicated that the secondary metabolites of the endophytic fungi extracts isolated from *C. officinalis* and *S. elongata* plants were more active in inhibiting the growth of *S. aureus*.

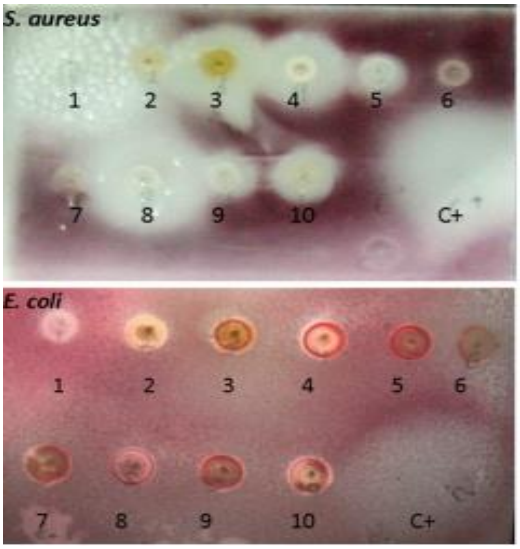


Figure 4. Bioautogram of the active antibacterial activity of endophytic fungi extracts against *S.aureus* and *E.coli*. Picture information refers to the Table 2.

The antibacterial activity of the endophytic fungi extracts was further quantified by evaluating the MIC value against *S. aureus* and *E. coli*. The lowest extract concentration that can inhibit bacterial growth is described as MIC. The results of the MIC of the endophytic fungi extracts were presented in Table 2.

The classification of antibacterial activity of the extract as follows: strong inhibitors (MIC<100 µg/ml), moderate

inhibitors (MIC 100-500 µg/ml), weak inhibitors (MIC 500-1000 µg/ml), and inactive (MIC>1000 µg/ml) [14], it can be concluded that all of the extracts presented weak activity against *E. coli*, while two extracts (CO_2 and SE3) exhibit significant antibacterial activity against *S. aureus*.

Table 2. The MIC of the endophytic fungi extracts.

No	Endophytic extracts	MIC (µg/ml)	
		S. aureus	*E. coli*
1	CO1	128	>256
2	CO2	<8	>256
3	SE3	<8	>256
4	CO4	>256	>256
5	CO5	256	>256
6	SE1	>256	>256
7	SE2	>256	>256
8	CO3	>256	>256
9	SE4	>256	>256
10	SE5	>256	>256
11	Chloramphenicol	4	4

This indicated that the endophytic fungi extracts were more effective against *S. aureus* than *E. coli*. The disparity in sensitivity between two types of Gram bacteria can be attributable to differences in the morphology and molecular components of membranes. Gram-negative bacteria tend to be less permeable to antibacterial agents than Gram-positive bacteria, due to the presence of the additional protection provided by the outer membrane layer containing lipopolysaccharides [21].

Antioxidant activity

TLC-bioautography on DPPH radical scavenging activity was carried out for the analysis of the antioxidant activity of endophytic fungi extracts associated with *C. officinalis* and *S. elongata* plants. The bioautogram of the antioxidant activity of endophytic fungi extracts was performed in Figure 5. The result of the antioxidant assay of endophytic fungi extracts with bioautography showed that some extracts had the antioxidant activity. It was indicated by the formation of the yellow spot on the purple background [22]. The DPPH assay is a common, rapid, simple, and low-cost method to measure the antioxidant activity [23].

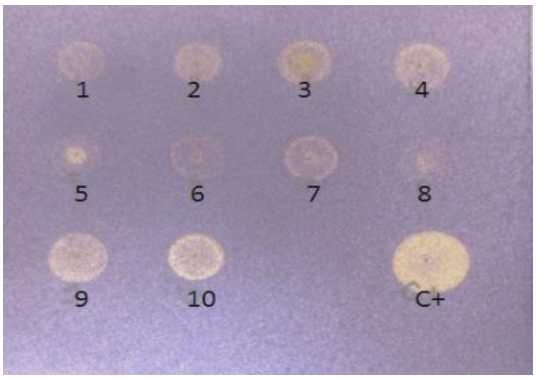


Figure 5. Bioautogram of antioxidant activity of endophytic fungi extracts. Extracts no. 1-10 are the extracts correspond to Table 3.

The antioxidant activity of the endophytic fungi extracts was further quantified by determining IC_{50} and AAI value. The amount of extract concentration required to produce a 50% reduction of DPPH free radical is defined as IC_{50} [24]. The results Table 3 showed that SE5 exhibited the highest antioxidant activity with an IC_{50} value of (56.3679 µg/ml).

The result on tabulated in Table 3 showed that only one extract (SE5) displayed a moderate antioxidants activity, while the remaining nine extracts showed poor antioxidant properties. The endophytic fungi extracts showed lower antioxidant activity than the positive control (+)-catechin.

This can be explained that (+)-catechin used is in the pure form. At the same time, the endophytic fungi extracts are the various mixtures chemical compounds, which not all the compounds have the antioxidant activity. The combination of chemical compounds in the extracts can neutralize, inhibit, or produce additive or synergistic effects by helping to increase the potential of the active compounds [25]TABLETA.

Table 3. Antioxidant Activity Index (AAI) and the IC_{50} value of the endophytic fungi extracts.

No	Extracts	IC_{50} (µg/ml)	AAI	Classification*
1	CO1	595.1189	0.0517	Poor
2	CO2	155.2049	0.1981	Poor
3	SE3	130.8974	0.2349	Poor
4	CO4	204.6893	0.1502	Poor
5	CO5	317.3946	0.0969	Poor
6	SE1	187.7699	0.1638	Poor
7	SE2	106.9311	0.2876	Poor
8	CO3	214.4872	0.1434	Poor
9	SE4	82.8305	0.3712	Poor
10	SE5	56.3679	0.5455	Moderate
11	Catechin	3.8700	7.9400	Strong

*classified as described by Scherer and Godoy, 2009 [15].

Therefore, it is necessary to isolate the chemical compounds responsible for bioactivity. The investigation of secondary metabolites contained in each extract needs to explore more to understand the diversity of secondary metabolites from isolated endophytic fungi with different host plants. Also, the understanding of secondary metabolites responsible for biological activity will trigger the discovery of effective antibiotic and antioxidant lead compounds.

ACKNOWLEDGMENTS

From this research, it was found two of the endophytic fungi, *Fusarium* sp. isolated from CO_2 and Hyphomycetes isolated from SE3, showed promising antimicrobial activity against *S. aureus* with MIC value <8 µg/ml. Only one endophytic fungi, *Colletotrichum* sp. isolated from SE5 that show a moderate antioxidant activity. This study showed the biological activity of endophytic fungi extracts isolated from medicinal plants. Further investigation of secondary metabolites contained in each extract needs to be addressed to know the effect of the host plant in the diversity of isolated secondary metabolites. Moreover, that also can be used to understand the secondary metabolite responsible for the biological activity that leads to the discovery of effective antibiotics and antioxidants.

ACKNOWLEDGMENTS

This research is supported by LIPI by DIPA scheme thorough special training for junior Researchers.

REFERENCES

[1] A. Alvin, K. I. Miller, and B. A. Neilan, “Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds,” *Microbiol. Res.*, vol. 169, no. 7–8, pp. 483–495, 2014, DOI:10.1016/j.micres.2013.12.009

[2] G. A. Strobel, “Endophytes as sources of bioactive products,” Microbes Infect., vol. 5, no. 6, pp. 535–544, 2003, DOI:10.1016/S1286-4579(03)00073-X.

[3] S. Mandal, M. Moudgil, and S. K. Mandal, "Rational drug design," Eur. J. Pharmacol., vol. 625, no. 1–3, pp. 90–100, 2009, DOI:10.1016/j.ejphar.2009.06.065.

[4] F. Chassagne, G. Cabanac, G. Hubert, B. David, and G. Marti, "The landscape of natural product diversity and their pharmacological relevance from a focus on the Dictionary of Natural Products ®," Phytochem. Rev., vol. 18, no. 3, pp. 601–622, 2019, DOI:10.1007/s11101-019-09606-2.

[5] D. J. Newman and G. M. Cragg, "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019.," J. Nat. Prod., 2020, DOI:10.1021/acs.jnatprod.9b01285.

[6] J. K. Stone, C. W. Bacon, and J. F. White, "An overview of endophytic microbes: endophytism defined," Microb. Endophytes, vol. 10, no. January 2000, pp. 3–29, 2000, DOI:10.1163/_q3_SIM_00374.

[7] S. K. Deshmukh, S. A. Verekar, and S. V. Bhave, "Endophytic fungi: A reservoir of antibacterials," Front. Microbiol., vol. 5, no. DEC, pp. 1–43, 2014, DOI:10.3389/fmicb.2014.00715.

[8] R. Nicoletti and A. Fiorentino, "Plant Bioactive Metabolites and Drugs Produced by Endophytic Fungi of Spermatophyta," Agriculture, vol. 5, no. 4, pp. 918–970, 2015, DOI:10.3390/agriculture5040918.

[9] M. I. Maulani, L. Purwanti, and U. A. Dasuki, "Uji Aktivitas Antibakteri Ekstrak Daun Reundeu [Staurogyne elongata (Bl.) O.K] terhadap Staphylococcus aureus dan Eschericia coli," Pros. Farm., vol. 3, no. 2, pp. 565–569, 2017, ISSN: 2460-6472. Google Scholar

[10] M. Ilyas and M. Rahmansyah, Seri Panduan : Teknik Isolasi Fungi. Jakarta: LIPI Press, 2006. Google Scholar

[11] M. Ilyas, A. Kanti, and M. Rahmansyah, Ilyas. Seri Panduan Teknik Preservasi Fungi. Jakarta: LIPI Press, 2007. Google Scholar

[12] M. Ilyas, Praptiwi, D. Wulansari, A. Fathoni, and A. Agusta, "An assemblages of fungal endophytes isolated from medicinal plants collected from Toba and Samosir, North Sumatra," IOP Conf. Ser. Earth Environ. Sci., vol. 308, no. 1, pp. 1–10, 2019, DOI:10.1088/1755-1315/308/1/012070.

[13] M. Raunsai, D. Wulansari, A. Fathoni, and A. Agusta, "Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi," vol. 8, no. 08, pp. 69–74, 2018, DOI:10.7324/JAPS.2018.8811.

[14] G. L. Passini, B. P. D. Filho, C. V. Nakamura, and D. A. G. Cortez, "Antibacterial Activity of Extracts and Neolignans from Piper regnellii (Miq.) C. DC. var. pallescens (C.DC.) Yunck," Mem Inst Oswaldo Cruz, vol. 98, no. 8, pp. 1115–1120, 2003, DOI:10.1590/s00740276200300080025.

[15] R. Scherer and H. T. Godoy, "Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method," Food Chem., vol. 112, pp. 654–658, 2009, DOI: 10.1016/j.foodchem.2008.06.026.

[16] M. Tanaka, H. Sukiman, M. Takebayashi, K. Saito, M. Suto, T. K. Prana, M. S. Prana, et al., "Isolation, Screening and Phylogenetic Identification of Endophytes from Plants in Hokkaido Japan and Java

Indonesia," Microbes Environ., vol. 14, no. 4, pp. 237–241, 1999, DOI: 10.1264/jsme2.14.237.

[17] B. Paulus, P. Gadek, and K. D. Hyde, "Estimation of microfungal diversity in tropical rainforest leaf litter using particle filtration: The effects of leaf storage and surface treatment," Mycol. Res., vol. 107, no. 6, pp. 748–756, 2003, DOI: 10.1017/S0953756203007913.

[18] Evana, Pratiwi, A. Fathoni, O. Efendi, and A. Agusta, "Antioxidant, Antibacterial Activity and GC-MS Analysis of Extract of Giant Forest Ant Dinomyrmex Gigas (Latreille, 1802)," J. Biodjati, vol. 4, no. 2, pp. 263–277, 2019, DOI: 10.15575/biodjati.v4i2.5440.

[19] M. T. G. Silva, S. M. Simas, T. G. F. M. Batista, P. Cardarelli, and T. C. B. Tomassini, "Studies on antimicrobial activity, in vitro, of Physalis angulata L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination," Mem Inst Oswaldo Cruz, vol. 100, no. 7, pp. 779–782, 2005, DOI:10.1590/s0074-02762005000700018.

[20] A. Adetutu, W. A. Morgan, and O. Corcoran, "Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extracts of Bridelia ferruginea leaf, a wound-healing plant of Nigeria," J. Ethnopharmacol., vol. 133, pp. 116–119, 2011, DOI: 10.1016/j.jep.2010.09.011.

[21] R. M. Epand, C. Walker, R. F. Epand, and N. A. Magarvey, "Molecular mechanisms of membrane-targeting antibiotics," BBA - Biomembr., vol. 1858, no. 5, pp. 980–987, 2016, DOI: 10.1016/j.bbamem.2015.10.018.

[22] J. Wang, Y.-D. Yue, F. Tang, and J. Sun, "TLC Screening for Antioxidant Activity of Extracts from Fifteen Bamboo Species and Identification of Antioxidant Flavone Glycosides from Leaves of Bambusa. textilis McClure," Molecules, vol. 17, pp. 12297–12311, Oct. 2012, DOI: 10.3390/molecules171012297.

[23] M. Brits and F. Bucar, "Antioxidant activity of Nigella sativa essential oil," Phytother. Res., vol. 14, no. 5, pp. 323–328, 2000 DOI:10.1002/10991573(200008)14:5

[24] P. Molyneux, "The use of the stable free radical diphenylpicryl-hydrazine (DPPH) for estimating antioxidant activity," Songklanakarin J. Sci. Technol., vol. 26, no. 2, pp. 211–219, 2004. Google Scholar

[25] S. Dhankhar, S. Kumar, S. Dhankhar, and J. P. Yadav, "Antioxidant Activity of Fungal Endophytes Isolated From Salvadora oleoides Decne," Int J Pharm Pharm Sci., vol. 4, no. 2, pp. 380–385, 2012, ISSN: 0975-1491. Google Scholar